

(51) International Patent Classification ⁵ : C07C 9/00, A61K 31/59		A1	(11) International Publication Number: WO 92/05130 (43) International Publication Date: 2 April 1992 (02.04.92)
(21) International Application Number: PCT/US91/06865 (22) International Filing Date: 20 September 1991 (20.09.91) (30) Priority data: 586,854 21 September 1990 (21.09.90) US (60) Parent Application or Grant (63) Related by Continuation US 586,854 (CIP) Filed on 21 September 1990 (21.09.90) (71) Applicant (for all designated States except US): LUNAR CORPORATION [US·US]: 313 West Behline Highway, Madison, WI 53713 (US).		(72) Inventors: and (75) Inventors/Applicants (for US only): KNUTSON, Joyce. C. [US/US]; 24 North Prospect Street, Madison, WI 53705 (US). BISHOP, Charles. W. [US/US]; 3641 Okanogan Court, Verona, WI 53573 (US). MORIARTY, Robert. M. [US/US]; 1030 Erie Street, Oak Park, IL 80302 (US). (74) Agents: GULBRANDSEN, Carl, E. et al.; 25 West Main Street, Suite 300, P.O. Box 2236, Madison, WI 53701-2236 (US). (81) Designated States: AT (European patent), AU, BE (European patent), BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LU (European patent), NL (European patent), NO, PL, SE (European patent), SU*, US.	
		Published With international search report.	
(54) Title: NOVEL 1 α -HYDROXY VITAMIN D ₃ AND NOVEL INTERMEDIATES AND ANALOGUES			
(57) Abstract <p>Novel 1α-hydroxy vitamin D₃ and novel analogues, 1,25 dihydroxy vitamin D₃ and 1,24 dihydroxy vitamin D₃ which are useful as active compounds of pharmaceutical compositions for the treatment of disorders of calcium metabolism. Preparation of the novel 1α-hydroxy vitamin D₃ starts from ergosterol which is converted in six steps to 22,23-dihydroergosterol. 22,23-dihydroergosterol was irradiated to yield vitamin D₃ which is converted in four steps to 1α-hydroxy vitamin D₃ using a cyclovitamin procedure which produces the novel intermediates, vitamin D₃ tosylate, 3,5 cyclovitamin D₃ and 1α-hydroxy cyclovitamin D₃. 1,25 dihydroxy vitamin D₃ and 1,24 dihydroxy vitamin D₃ are isolated as biological products of the metabolism of novel 1α-hydroxy vitamin D₃ using cultured human liver cells.</p>			

NOVEL 1α -HYDROXY VITAMIN D_4
AND NOVEL INTERMEDIATES AND ANALOGUES

TECHNICAL FIELD

This invention relates to biologically active vitamin D_4 compounds. More specifically, this invention relates to novel 1α -hydroxy vitamin D_4 and novel intermediates used in its synthesis, novel 1,25 dihydroxy vitamin D_4 , and novel 1,24 dihydroxy vitamin D_4 .

This invention also relates to a pharmaceutical composition which includes a pharmaceutically effective amount of the novel 1α -hydroxy vitamin D_4 compounds, and to a method of controlling abnormal calcium metabolism by administering a pharmaceutically effective amount of the novel compounds.

BACKGROUND

Vitamin D is known to be important in the regulation of calcium metabolism in animals and man. See, Harrison's Principals of Internal Medicine: Part Eleven, "Disorders of Bone and Mineral Metabolism, Chapter 335," E. Braunwald, et al., (eds.), McGraw-Hill, New York, 1987, pp. 1860-1865. The two most commonly known, useful forms of vitamin D are vitamin D_3 and vitamin D_2 . Vitamin D_3 is synthesized endogenously in the skin of animals and man, whereas vitamin D_2 is the form of vitamin D supplied by plants. Vitamin D_2 differs from vitamin D_3 in that it contains a double bond between C22 and C23 and further contains a C24-methyl group. In man and rats, vitamin D_3 and vitamin D_2 have equivalent biopotency.

Vitamin D_4 , also known as irradiated 22,23-dihydro-ergosterol or 22,23-dihydro vitamin D_2 or 22,23-dihydroergocalciferol, differs from vitamin D_3 in that it contains a C24 methyl group. Vitamin D_4 was first described in 1936. See, Grab, W., Z. Physiol. Chem., 243:63 (1936); McDonald, F.G., J. Biol. Chem., 114:IVX (1936). See also, Windaus, A. and Trautmann, G., Z. Physiol. Chem., 247:185-188 (1937). These references report some disagreement as to the level of biological activity of the vitamin suggesting that in the rat, vitamin D_4 is one-third or three-fourths as active as vitamin D_3 .

therapeutic agent.

SUMMARY OF THE INVENTION

The novel compounds of the invention, 1 α -hydroxy vitamin D₃, 1,25-dihydroxy vitamin D₃ and 1,24-dihydroxy vitamin D₃, are bioactive forms of vitamin D₃. The present inventors have discovered that these active forms of vitamin D₃ display much greater biopotency than would be predicted on the basis of the previously reported bioassays of vitamin D₃. The present inventors have also discovered, that the bioactive novel compounds are less toxic than would be predicted on the basis of their biopotency. This combination of high activity with low toxicity makes the compounds of the invention useful as therapeutic agents in the treatment of disorders of calcium metabolism. The novel compounds of the invention are advantageously used as the active compounds of pharmaceutical compositions for diseases induced by abnormal metabolism of calcium.

In order to study the novel compounds of the invention, it was necessary to develop processes for their production. One alpha-hydroxy vitamin D₃ was made synthetically and in the course of that synthesis, novel intermediates were also produced. 1,25-dihydroxy vitamin D₃ and 1,24-dihydroxy vitamin D₃ are isolated as biological products of the metabolism of 1 α -hydroxy vitamin D₃.

Other advantages and a fuller appreciation of the specific adaptations, compositional variations, and physical and chemical attributes of the present invention will be gained upon an examination of the following detailed description of the invention, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will hereinafter be described in conjunction with the appended drawings, wherein like designations refer to like elements throughout and in which:

Figure 1 illustrates preparative steps for the synthesis of vitamin D₃; and

Figure 2 illustrates preparative steps for the synthesis of 1 α -hydroxy vitamin D₃ starting with vitamin D₃.

four-step process to yield 1 α -hydroxy vitamin D₄ using a procedure similar to that described by Paaren, et al., J. Org. Chem., 1980, 45:3253.

Specifically, ergosterol is acetylated to form the 3 β -acetate. This ergosterol acetate is subjected to hydroxyhalogenation at the 5,6 double bond to form the 6 α -chloro-5 α -hydroxy derivative. This chlorohydrin is reduced and reacetylated to the 5 α -hydroxy (i.e., 5 α -ol) derivative. The 5 α -ol is subjected to hydrogenation to saturate the side chain. The resulting 3 β -acetoxysterol-7en-5 α -ol is reduced to 22,23 dehydroergosterol acetate which is in turn reduced to yield 22,23 dehydroergosterol. The 22,23 dehydroergosterol is then irradiated to form vitamin D₄. Vitamin D₄ is then tosylated to yield 3 β -tosyl vitamin D₄. The tosylate is displaced by solvolysis to yield the 6-methoxy-3,5-cyclovitamin D₄. The cyclovitamin D₄ is subjected to allylic oxidation to form the 1 α -hydroxy cyclovitamin derivative. The 1 α -hydroxy cyclovitamin derivative is sequentially solvolyzed and subjected to a Diels-Alder-type reaction which removes the 5-methoxy group and separates the 1 α -hydroxy vitamin D₄ (5,6-cis) from the 5,6 trans-1 α -hydroxy vitamin D₄.

The 1,24 dihydroxy vitamin D₄ and 1,25 dihydroxy vitamin D₄ metabolites of 1 α -hydroxy vitamin D₄, are synthesized by incubating the 1 α -hydroxy derivatives with human liver cells, culturing the cells, and recovering the 1,24 dihydroxy or 1,25 dihydroxy vitamin D₄. Using vitamin D receptor protein binding tests, these metabolites are determined to be biologically active.

The compounds of formula (I) have been found to possess valuable pharmacological activity, namely, as controlling agents for calcium metabolism, especially serum calcium concentrations. Specifically, the compounds of formula (I) increase serum calcium concentrations in rats with vitamin D deficiency. It has also been found that the compounds of formula (I) have low toxicity, which enhances their pharmaceutical properties. Compounds of formula (I) have a toxicity, as measured by the LD₅₀ test, which is similar to that of corresponding vitamin D₂ compounds and lower than that of corresponding vitamin D₃ compounds. Thus, the compounds of the invention are applicable

vegetable oils (e.g., corn oil, cottonseed oil, peanut oil, olive oil, coconut oil), fish liver oils, oily esters such as Polysorbate 80, polyethylene glycols, gelatine, carbohydrates (e.g., lactose, amylose or starch), magnesium stearate, talc, silicic acid, viscous paraffin, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc.

The pharmaceutical preparations can be sterilized and, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, vitamin D₃ or D₂ and their 1 α -hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalamin, pertussis toxin and boron.

For parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solution, as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, lozenges, powders, or capsules. A syrup, elixir, or the like can be used if a sweetened vehicle is desired.

Sustained or directed release compositions can also be formulated, e.g., liposomes or those in which the active compound is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

For topical application, suitable nonsprayable viscous, semi-solid or solid forms can be employed which include a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable formulations include, but are not limited to, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, aerosols, transdermal patches, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, demulsifiers, wetting agents, etc.

For rectal administration, compounds are formed into a pharmaceutical composition containing a suppository base such as

3000 Computer in CDCl_3 solutions with CHCl_3 as an internal standard. Infrared spectra were recorded with a Fourier transform (FTIR) using samples as potassium bromide (KBr) pellets or as liquids. Mass spectra were recorded with a Finnigan MAT-90 mass spectrometer at 20 eV/CI. Melting points are determined on a Hoover-Thomas (capillary) Uni-Melt and a Fisher-Johns melting point apparatus (cover-slip type).

Example 1: Synthesis of 1 α -hydroxy vitamin D₄

Ergosterol (II) was converted to ergosterol acetate (III) by dissolving 100 g (0.25 mol) ergosterol in 600 ml of anhydrous pyridine and 68 ml (0.7 mol) acetic anhydride. The solution was stirred overnight at room temperature after which time the solution was cooled by adding 1.2 L ice, causing a precipitate to form. The precipitate was washed five times with 400 ml portions of water, then once with 400 ml of CH_3CN . The resulting product was air dried to yield 79 g (71%) of ergosterol acetate as a white crystalline solid and had the following characteristics: melting point (m.p.): 169-171°C; ^1H NMR: (400 MHz, CDCl_3), δ ppm 2.05 (3H, s, 3 β - CH_3CO), 4.65-4.75 (1H, m, 3 α -H), 5.15-5.25 (2H, m, 22-H and 23-H), 5.4 (1H, d, 6-H), 5.6 (1H, d, 7-H); FTIR [KBr]: 1734 cm^{-1} (C=O stretching) 968 cm^{-1} (C-H bending).

Ergosterol acetate (III) (26 gm, 0.062 M) was dissolved in 2.5 L of freshly distilled deoxygenated toluene. To this solution 9 ml (0.111 mol) chromyl chloride dissolved in 240 ml dry CH_2Cl_2 was added under nitrogen at -78°C over a thirty minute period. The reaction system was stirred at -78°C for an additional fifteen minutes, and then 62 ml of a saturated solution of sodium borohydride in ethanol was added in one portion. After stirring at -78°C for an additional fifteen minutes, the reaction solution was poured into a two phase system of 3N hydrochloric acid (3L) and benzene (3L). The organic layer was separated, then washed with water (2L), twice with a brine solution (2 x 1L) and then dried with anhydrous MgSO_4 . The dried solution was filtered and concentrated in vacuo. The crude crystalline product was then treated with CH_3CN (280ml) and filtration of the thus formed slurry yielded 12.5 g (41%) of white crystalline 3 β -Acetoxy-6 α -chloroergosta-7,22-dien-5 α -ol

was converted to a slurry with CH_3CN (100 ml). The product was collected by filtration and recrystallized from CH_3CN to yield 4.5 g. (39%) of a white crystalline 22,23-dihydroergosteryl acetate (VII) and had the following characteristics: m.p.: 144-147°C; ^1H NMR: (400 MHz, CDCl_3), δ ppm 2.05 (3H, s, 3 β -OAc), 4.65-4.75 (1H, m, 3 α -H), 5.4 (1H, d, 6-H), 5.6 (1H, d, 7-H); FTIR [KBr]: 1734 cm^{-1} (C=O stretching).

22,23-dihydroergosteryl acetate (VII) (4.8 g, 0.011 mol) was added at once to a stirred suspension of lithium aluminium hydride (2.5 g, 0.066 mol) in dry ether (1.1 L) at room temperature. The mixture was stirred for two hours at room temperature. 5N NaOH was added to destroy excess lithium aluminium hydride and H_2O (500 ml) was then added. The aqueous solution was then extracted with four 250 ml portions of ether. The combined ether extracts and combined organic layer were washed with brine solution (1 L), then dried with Na_2SO_4 . Evaporation of ether under reduced pressure gave the compound, 22,23-dihydroergosterol, (VIII) (4.1 g, 94%) as a white crystalline material and had the following characteristics: m.p.: 147-150°C; ^1H NMR: (400 MHz, CDCl_3), δ ppm 3.6-3.7 (1H, m, 3 α -H), 5.4 (1H, d, 6-H), 5.6 (1H, d, 7-H); FTIR [KBr]: 3400 cm^{-1} (O-H stretching).

22,23-dihydroergosterol (VIII) (2.0 g, 5.0 mmol) was dissolved in a solution of diethyl ether and benzene (4:1, 600 ml) and irradiated (Hannovia immersion lamp, 450 watts) with stirring under argon in a water-cooled quartz vessel for three hours. The solution was concentrated in vacuo to yield a gummy solid, which was redissolved in 100 ml. of ethanol and heated at reflux under argon for eight hours. Then, the solution was concentrated in vacuo and the residue was adsorbed on a silica gel column and eluted with 30% ethyl acetate in hexane to afford vitamin D_4 (22,23-dihydroergocalciferol) (IX) with a yield of 1.2 g. (60%) and with the following characteristics: ^1H NMR: (400 MHz, CDCl_3), δ ppm 0.55 (3H, s, 18- H_3) 0.78 (6H, dd, 26- H_3 and 27- H_3) 0.87 (3H, d, 21- H_3) 0.93 (3H, d, 28- H_3) 3.94 (1H, m, 3-H) 4.82 (1H, m (sharp), 19-H), 5.04 (1H, m (sharp), 19-H), 6.04 (1H, d, 7-H) 6.24 (1H, d, 6-H).

To a stirred solution of vitamin D_4 (IX) (3.0 g, 7.5 mmol) in 10 ml of dry pyridine was added freshly recrystallized p-

saturated NaCl solution (2 x 200 ml), dried over MgSO_4 and concentrated in vacuo. The residue was absorbed on a silica gel column and eluted with 30% ethyl acetate in hexane to afford 0.45 g. (29%) of the novel intermediate compound 1 α -hydroxy 3,5-cyclovitamin D_4 (XII) (oil) and had the following characteristics: ^1H NMR (400 MHz, CDCl_3), δ ppm 0.54 (3H, s, 18- H_3) 0.78 (6H, dd, 26- H_3 and 27- H_3) 0.86 (3H, d, 21- H_3) 0.95 (3H, d, 28- H_3) 3.26 (3H, s, OCH_3) 4.2 (1H, d, 6-H), 4.22 (1H, m, 1-H), 4.95 (1H, d, 7-H), 5.18 (1H, d, 19-H) 5.25 (1H, d, 19-H).

A solution of 1 α -hydroxy 3,5-cyclovitamin D_4 (XII) (0.45 g, 1.05 mmol) in a solution of dimethyl sulfoxide (4.5 ml) and glacial acetic acid (3.6 ml) was heated to 50°C under argon for one hour. The reaction mixture was then poured over ice and saturated NaHCO_3 solution (100 ml), and extracted with ether (3 x 200 ml). The combined ether extracts were washed with saturated NaHCO_3 solution (3 x 200 ml), water (3 x 200 ml) and saturated NaCl solution (3 x 200 ml), dried over MgSO_4 , concentrated in vacuo to give a mixture containing 5,6-cis and 5,6-trans 1 α -hydroxy vitamin D_4 (about 4:1 by ^1H NMR) with a yield of 0.4g, (92%). The mixture of 5,6-cis and 5,6-trans 1 α -hydroxy vitamin D_4 (0.4 g, 0.97 mmol) was dissolved in ethyl acetate (25 ml) and treated with freshly recrystallized maleic anhydride (0.08 g, 0.8 mmol). This reaction mixture was heated to 35°C under argon for 24 hours. After evaporation of the solvent in vacuo, the crude mixture was chromatographed over a silica gel column using ethyl acetate and hexane (1:1) as eluent, to afford the novel active form of vitamin D_4 , 5,6-cis 1 α -hydroxy vitamin D_4 (XIII) with a yield of 90 mg (23%) and had the following characteristics: m.p.: 128-130°C; IR ν_{max} (Neat): 3400 cm^{-1} (OH stretching); ^1H NMR (400 MHz, CDCl_3), δ ppm 0.55 (3H, s, 18-H) 0.79 (6H, dd, 26- H_3 and 27- H_3) 0.87 (3H, d, 21- H_3) 0.94 (3H, d, 28- H_3), 4.24 (1H, m, 3-H), 4.44 (1H, m, 1-H), 5.02 (1H, m (sharp), 19-H), 5.34 (1H, m (sharp), 19-H), 6.02 (1H, d, 7-H), 6.4 (1H, d, 6-H); Mass spectrum [CI] m/e (relative intensity): 415 ($\text{M}+1$, 41%) 397, ($\text{M}+1-\text{OH}$ 100%), 379 (27%), 135 (22%).

Example 2: Biological testing of 1 α -hydroxy vitamin D_4

Male weanling rats (Holtzman strain, Holtzman Company, Madison, Wisconsin) were fed a vitamin D deficient diet

method. Rats were fed a standard laboratory diet for 8-10 weeks. Five animals of each sex were administered one oral dose of 1α -OH- D_4 . The animals were observed for 14 days, and the number of deaths noted. The LD_{50} value was determined to be about 1.0 mg/kg in males and 3.0 mg/kg in females.

For comparison, the LD_{50} value for 1α -hydroxy vitamin D_2 under the same conditions was found by applicant's to be 1.7 and 1.8 mg/kg. in male and female rats, respectively. The toxicity of 1α -hydroxy vitamin D_2 has previously been reported as less than 1α -hydroxy vitamin D_3 . Sjoden, G., Smith, C., Lindgren, U., and DeLuca, H.P., Proc. Soc. Experimental Biol. Med., 178:432-436 (1985).

Example 4: Generation and Isolation of 1,25-dihydroxy vitamin D_4

The 1α -hydroxy vitamin D_4 of the present invention is incubated with cultured human liver cells which metabolize the compound to several products including the metabolite 1,25 dihydroxy vitamin D_4 . The 1,25 metabolite is isolated and purified by high pressure liquid chromatography and identified by gas-chromatography-mass spectrometry. Binding studies demonstrate that the 1,25 dihydroxy vitamin D_4 has good binding affinity for the mammalian vitamin D receptor protein indicating it is biologically active. The procedures used are similar to that described by Strugnell, et. al., Biochem. Pharm. Vol. 40:333-341 (1990).

Example 5: Generation and isolation of 1,24-dihydroxy vitamin D_4

Generation and isolation of 1,24 dihydroxy vitamin D_4 is accomplished as described in Example 4, above. The 1α -hydroxy vitamin D_4 of the present invention is incubated with cultured human liver cells which metabolize the compound to several products including the metabolite 1,24 dihydroxy vitamin D_4 . The 1,24 metabolite is isolated and purified using high pressure liquid chromatography and identified by gas-chromatography-mass spectrometry. Binding studies with the new metabolite demonstrate that the metabolite has good binding affinity for the mammalian vitamin D receptor protein which indicates the drug is biologically active.

comparisons of urinary hydroxyproline excretion, serum and urine calcium levels, creatinine clearance, blood urea nitrogen, and other routine determinations.

This study demonstrates that patients treated with 1α -vitamin D_3 exhibit significantly higher total body, radial, femoral and/or spinal bone densities relative to patients treated with placebo. The treated patients also exhibit significant elevations in serum osteocalcin. Bone biopsies from the treated patients show that 1α -vitamin D_3 stimulates normal bone formation. The monitored safety parameters confirm an insignificant incidence of hypercalcemia or hypercalciuria, or any other metabolic disturbance with 1α -vitamin D_3 therapy.

Example 9:

A clinical study is conducted with healthy postmenopausal women having ages between 55 and 60 years. The study involves up to 80 patients randomly divided into two treatment groups, and continues for 12 to 24 months. One treatment group receives a constant dosage of 1α -vitamin D_3 (u.i.d.; a dose level above $3.0 \mu\text{g/day}$) and the other receives a matching placebo. The study is conducted as indicated in Example 2 above.

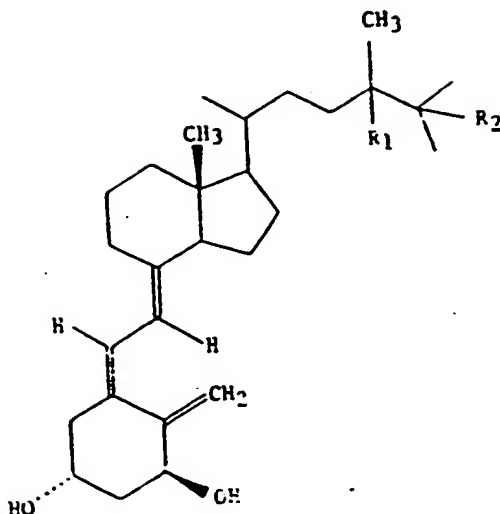
This study demonstrates that patients treated with 1α -vitamin D_3 exhibit reduced losses in total body, radial, femoral and/or spinal bone densities relative to baseline values. In contrast, patients treated with placebo show significant losses in these parameters relative to baseline values. The monitored safety parameters confirm the safety of long-term 1α -vitamin D_3 administration at this dose level.

Example 10:

A twelve-month double-blind placebo-controlled clinical trial is conducted with thirty men and/or women with renal disease who are undergoing chronic hemodialysis. All patients enter an eight-week control period during which time they receive a maintenance dose of vitamin D_3 (400 IU/day). After this control period, the patients are randomized into two treatment groups: one group receives a constant dosage of 1α -vitamin D_3 (u.i.d.; a dosage greater than $3.0 \mu\text{g/day}$) and the other group receives a matching placebo. Both treatment groups

CLAIMS:

1. The compound of the formula (I):



(I)

wherein R₁ is either H or OH and R₂ is either H or OH and salts, hydrates and solvates thereof.

2. The compound of claim 1, wherein said compound is 1 α -hydroxy vitamin D₄.

3. The compound of claim 1, wherein said compound is 1,24 dihydroxy vitamin D₄.

4. The compound of claim 1, wherein said compound is 1,25 dihydroxy vitamin D₄.

5. The compound of claim 1, wherein said compound is biologically active.

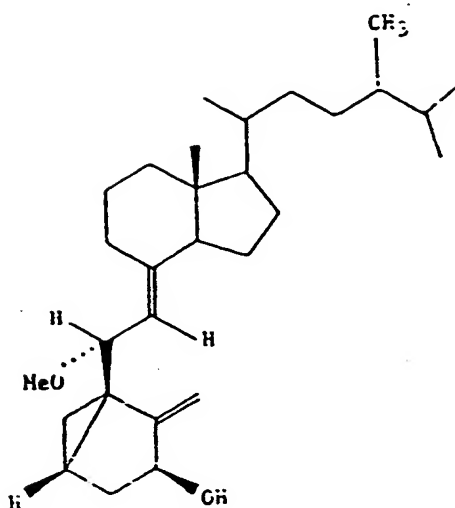
6. The compound of formula (I) according to claim 1, wherein R₁ is H or OH and R₂ is H or OH and wherein said compound exhibits biological activity approaching that of 1,25 vitamin D₃ and wherein said compound is less toxic than 1 α -hydroxy vitamin D₃ as determined by comparative LD₅₀ values in rats.

7. The compound of claim 6, wherein said compound is 1 α -hydroxy vitamin D₄.

8. The compound of claim 6, wherein said compound is 1,25 dihydroxy vitamin D₄.

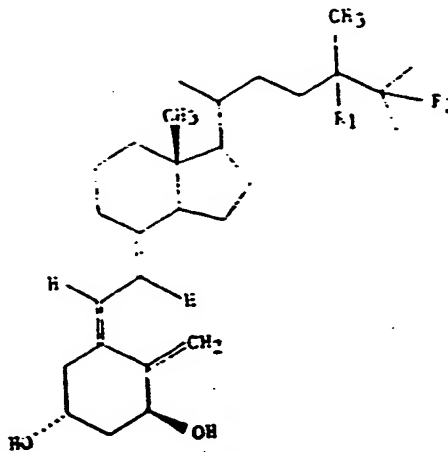
9. The compound of claim 6, wherein said compound is 1,24 dihydroxy vitamin D₄.

12. The 1 α -hydroxy 3,5 cyclovitamin D₄ of the formula (XII):



(XII)

13. A pharmaceutical composition, comprising an amount effective to increase serum calcium in a patient suffering vitamin D deficiency of a compound of the formula (I):



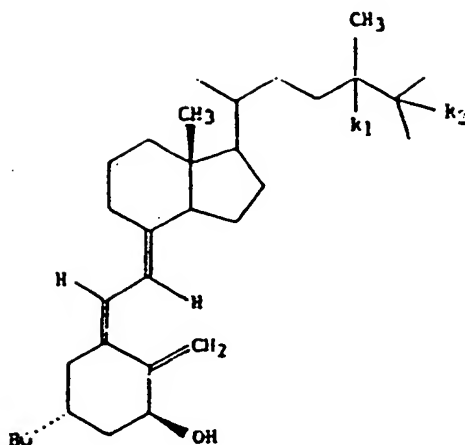
(I)

wherein R₁ is either H or OH and R₂ is either H or OH in combination with a pharmaceutically acceptable vehicle.

14. The pharmaceutical composition of claim 13, wherein said amount is administered orally.

15. A method of treating vitamin D deficiency induced diseases comprising administering to a patient suffering

increase serum calcium in the mammal, of a compound having the formula (I):



(I)

wherein R₁ is either H or OH and R₂ is either H or OH.

18. The method of claim 17, wherein said mammal suffers a vitamin D deficiency.

(19) The method of claim 17, wherein said compound is administered in a daily dose of about 0.04 µg to about 1.5 µg per kg of body weight of the treated mammal.

20. The method of claim 17, wherein the hypocalcemia is vitamin D dependent rickets, hypoparathyroidism, post-operative renal osteodystrophy, liver cirrhosis, or steatorrhea.

21. A method of producing vitamin D₄ tosylate, comprising reacting vitamin D₄ with toluenesulfonyl chloride in the presence of dry pyridine.

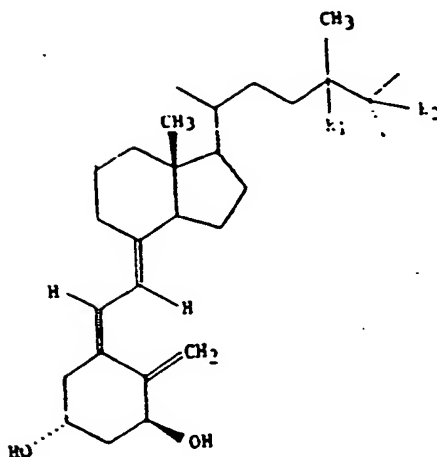
22. A method of producing 3,5 cyclovitamin D₄, comprising subjecting vitamin D₄ tosylate to buffered solvolysis.

23. A method of producing 1α-hydroxy 3,5 cyclovitamin D₄, comprising allylically oxidizing the 3,5 cyclovitamin D₄ with selenium dioxide.

24. A method of producing 1α-hydroxy vitamin D₄, comprising solvolizing the 1α-hydroxy 3,5 cyclovitamin D₄ with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 cis 1α-hydroxy and 5,6 trans 1α-hydroxy vitamin D₄ and subjecting the admixture to a Diels-Alder reaction forming an adduct of the 5,6 trans 1α-hydroxy vitamin D₄ to yield the 1α-hydroxy vitamin D₄.

25. A method of producing 1α-hydroxy vitamin D₄,

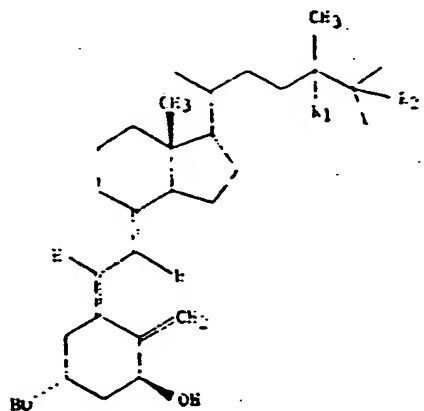
an effective amount of at least one compound of formula (I):



(I)

wherein R1 is either H or OH and R2 is either H or OH.

(28) A prophylactic or therapeutic pharmaceutical composition for vitamin D deficient diseases, comprising a physiologically acceptable vehicle and an effective amount of at least one compound of formula (I):

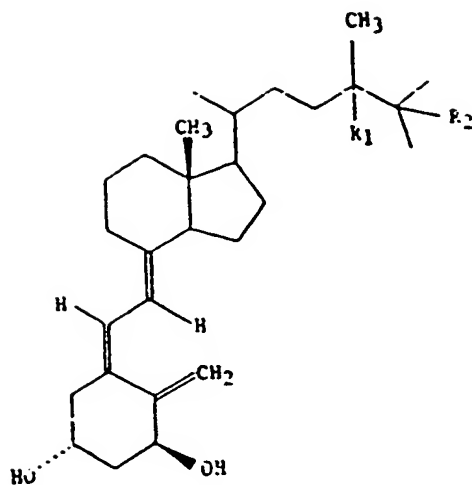


(I)

wherein R1 is either H or OH and R2 is either H or OH.

29. A method of controlling calcium metabolism in a mammal, comprising administering to a mammal a pharmaceutically

formula (I):



(I)

wherein R_1 is either H or OH and R_2 is either H or OH in combination with a pharmaceutically acceptable vehicle.

34. A method for treating vitamin D deficiency-induced hypocalcemia, comprising:

- (a) reducing ergosterol, under such conditions and in sufficient quantity to produce 22,23 dihydroergosterol;
- (b) irradiating the 22,23 dihydroergosterol to produce vitamin D_4 ;
- (c) hydroxylating the vitamin D_4 under such conditions and in sufficient quantity to produce 1 α -hydroxy vitamin D_4 ;
- (d) purifying the vitamin D_4 ; and
- (e) administering to a mammal suffering from vitamin D deficiency-induced hypocalcemia an amount effective to increase serum calcium of 1 α -hydroxy vitamin D_4 in admixture with a pharmaceutically acceptable vehicle.

35. A pharmaceutical composition for treating osteoporosis comprising a physiologically acceptable vehicle and an effective

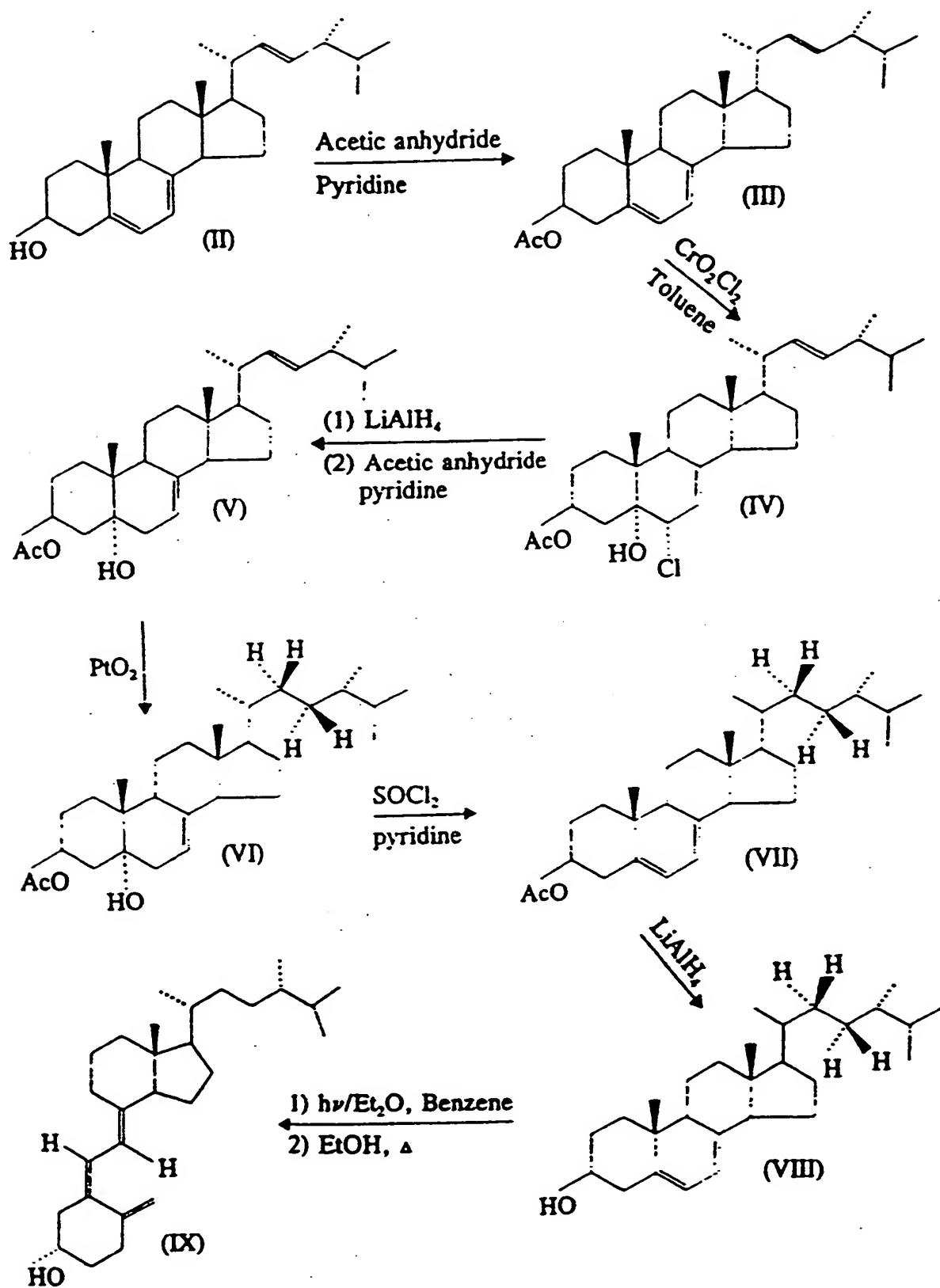


FIGURE 1

SUBSTITUTE SHEET

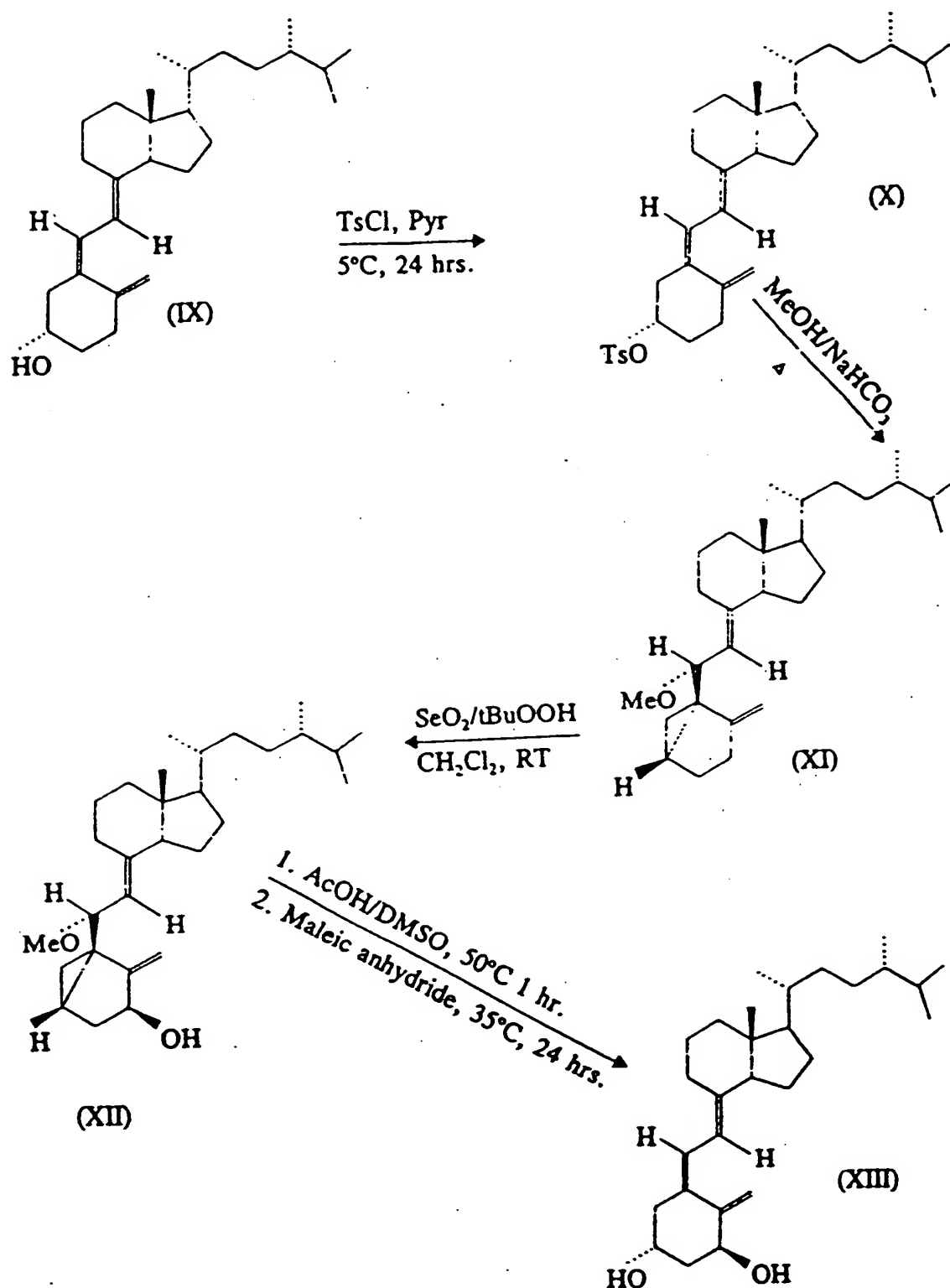


FIGURE 2

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

Application No. PCT/US91/06862

1. CLASSIFICATION OF SUBJECT MATTER

IPC(5): C07C 9/00, A61K 31/59
US CL 552/653, 514/168

2. FIELDS SEARCHED

U.S. 552/653, 514/168

Documentation Searched other than Minimum Documentation
To the Extent that such Documents are Included in the Fields Searched

3. DOCUMENTS CONSIDERED TO BE RELEVANT

Category of Document: Citation of Document: Information where appropriate of the relevant passages: Relevance to Claim No.

- | | | |
|---|--|-------------------------------------|
| Y | US, A, 4,202,859 (DULUCA ET AL.) 13 MAY 1980 | 1-9,13-15,
17-20,27-31,
33-36 |
| Y | DELUCA ET AL. Arch. Biochem. and biophys. 124, 122-128 (1965) Synthesis, Biological Activity and Metabolism of 22,23 ³ H Vitamin D ₄ . | 1-9,13-15,17-20,27-31,33-36 |
| Y | Windows, et al. 2. Physiol. Chem. 247, 1937, pp. 185 to 188. Uber das Krystallisierte Vitamin D ₄ . | 1-9,13-15,17-20,27-31,33-36 |

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the invention but cited to understand the principle or theory underlying the invention

- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

- "Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

25 OCTOBER 1991

International Searching Authority

ISA/US

Date of Mailing of the International Search Report

20 DEC 1991

Signature of Authorized Officer

Mukund J. Shah

FURTHER INFORMATION CONTINUED FROM THE FIRST SHEET
 (Not to be used for publication)

Group V, Claims 25, 2nd process of preparing vitamin D.
 Group VI, Claim 26, 3rd process method of preparing vitamin D.
 Group VII, Claim 32, animal feed.

THIS PAGE BLANK (USPTO)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ BLACK BORDERS

☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

☐ FADED TEXT OR DRAWING

☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING

☐ SKEWED/SLANTED IMAGES

☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS

☐ GRAY SCALE DOCUMENTS

☒ LINES OR MARKS ON ORIGINAL DOCUMENT

☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)